

Claims:

1. A method for generating a woody perennial breeding line, comprising:
 - a) selecting one or more woody perennial plants comprising at least one allele associated with male sterility;
 - 5 b) selecting one or more woody perennial plants which are capable of hybridisation with the plant(s) selected in step (a) and which comprise at least one allele associated with at least one target inheritable trait;
 - c) crossing the one or more plants selected in step (a) with the one or more plants selected in step (b);
 - 10 d) selecting progeny plants which have one or more desired traits and which comprise at least one allele associated with male sterility and at least one allele associated with the at least one target trait;
 - e) selecting progeny plants which are homozygous for male sterility, which comprise at least one allele associated with the at least one target trait and which have one
15 or more desired traits.
2. The method of claim 1, wherein step (d) further comprises allowing at least one of the selected F₁ progeny plants which is heterozygous for male sterility to self-fertilise to create an F₂ progeny for further selection.
3. The method of claim 1, wherein the flowers of one or more of the F₁
20 progeny are fertilised with a mixture of pollen obtained from a plurality of the selected F₁ progeny.
4. The method of claim 1, wherein step (d) further comprises crossing at least one of the selected F₁ progeny plants with one or more woody perennial plants of known genotype with respect to a desired set of inheritable traits and which comprises at least
25 one allele associated with male sterility to create an F₂ progeny for further selection.
5. The method of any one of claims 1 to 4, wherein the one or more woody perennial plants selected in step (a) are male sterile.
6. The method of claim 5, wherein the one or more woody perennial plants of
step (a) are grown in an isolated block, with the one or more woody perennial plants of
30 step (b).
7. The method of any one of claims 1 to 6, wherein a plurality of woody perennial plants are selected in step (a), and the flowers of the one or more woody perennial plants selected in step (b) are fertilised with a mixture of pollen collected from the woody perennial plants of step (a).

8. The method of any one of claims 1 to 6, wherein a plurality of woody perennial plants are selected in step (b), and the flowers of the one or more woody perennial plants selected in step (a) are fertilised with a mixture of pollen collected from the woody perennial plants of step (b).

5 9. The method of any one of claims 6 to 8, wherein the plurality of woody perennial plants selected in step (a), step (b) or both comprise more than one variety of woody perennial plant.

10 10. The method of any one of claims 1 to 9, wherein one woody perennial plant is selected in step (a), step (b) or both.

11. The method of any one of claims 1 to 10, wherein the one or more woody perennial plants selected in step (a) are of known genotype for a set of desired inheritable traits.

12. The method of any one of claims 1 to 11, wherein the one or more woody perennial plants selected in step (a) express a set of desired inheritable traits.

15 13. The method of any one of claims 1 to 11, wherein the one or more woody perennial plants selected in step (a) are homozygous for one or more desired inheritable traits.

20 14. The method of any one of claims 1 to 11, wherein the one or more woody perennial plants selected in step (a) are homozygous for each trait within a set of desired inheritable traits.

15. The method of any one of claims 1 to 14, wherein the one or more woody perennial plants of step (b) are of known genotype for a set of desired inheritable traits in addition to the target trait.

25 16. The method of any one of claims 1 to 15, wherein the one or more woody perennial plants of step (b) express a set of desired inheritable traits.

17. The method of any one of claims 1 to 16, wherein the one or more woody perennial plants of step (b) are homozygous for one or more desired inheritable traits.

30 18. The method of any one of claims 1 to 17, wherein the one or more woody perennial plants of step (b) are homozygous for each trait within a set of desired inheritable traits.

19. The method of any one of claims 1 to 18, wherein one or more of the plants of step (b) are heterozygous for male sterility.

20. The method of any one of claims 1 to 19, wherein all of the woody perennial plants are of the same species.

21. The method of any one of claims 1 to 19, wherein the one or more woody perennial plants of step (a) are of a different species to the one or more woody perennial plants of step (b).

22. The method of any one of claims 1 to 19, wherein the one or more woody perennial plants of step (a) are of a different genus to the one or more woody perennial plants of step (b).

23. The method of claim 21 or claim 22, wherein the ability of the woody perennial plants of steps (a) and (b) to hybridise is determined by cladistic analysis.

24. The method of claim 23, wherein the cladistic analysis comprises DNA extraction, electrophoresis and DNA banding analysis.

25. The method of any one of claims 1 to 24, wherein the one or more plants of step (b) are derived from a plant comprising fewer chromosomes than the plant of step (a) by increasing its ploidy.

26. The method of claim 25, wherein a plurality of plants are selected in step (a), step (b), or both, and a mixture of pollens from these plants are employed in the cross of step (c).

27. The method of any one of claims 1 to 26, wherein step (d) further comprises one or more sequential back-crosses of one or more selected progeny plants with one or more woody perennial plants of known genotype with respect to a desired set of inheritable traits, and selecting resulting progeny plants which comprise at least one allele coding for male sterility and at least one allele associated with the at least one target trait and which are of known genotype for the desired set of inheritable traits.

28. The method of claim 4 or claim 27, wherein the one or more parental woody perennial plants used for backcrossing to the progeny of step (d) express a set of desired inheritable traits.

29. The method of any one of claims 4, 27 or 28, wherein the one or more parental woody perennial plants used for backcrossing to the progeny of step (d) are homozygous for one or more desired inheritable traits.

30. The method of any one of claims 4, or 27 to 29, wherein the one or more parental woody perennial plants used for backcrossing to the progeny of step (d) are homozygous for each trait within a set of desired inheritable traits.

31. The method of any one of claims 4, or 27 to 30, wherein the one or more parental woody perennial plants used for backcrossing to the progeny of step (d) are heterozygous for male sterility.

32. The method of any one of claims 4, 27 to 31, wherein the one or more parental woody perennial plants used for backcrossing to the progeny of step (d) are of the same species as the one or more woody perennial plants of step (a).

33. The method of any one of claims 4, or 27 to 32, wherein the one or more
5 parental woody perennial plants are of the same species as the one or more woody perennial plants of step (b).

34. The method of any one of claims 4, or 27 to 31, wherein the one or more parental woody perennial plants used for backcrossing to the progeny of step (d) are the same, or are of the same variety as the woody perennial plant of step (a).

10 35. The method of any one of claims 4, or 27 to 31, wherein the one or more parental woody perennial plants used for backcrossing to the progeny of step (d) are the same, or are of the same variety as the one or more woody perennial plants of step (b).

36. The method of any one of claims 1 to 35, wherein the at least one target trait is selected from the group comprising: low chill requirement; high chill requirement;
15 disease/pest resistance; fruit development period; fruit acidity; fruit shape; fruit size; fruit flesh texture; fruit total solids (sugars); fruit pigmentation; fruit flesh pigmentation; fruit skin pubescence; stone adhesion to the fruit; tree habit; tree size; tree growth rate; spur morphology/ habit; pedicel length; pedicel thickness; suture presence/absence.

37. The method of claim 36, wherein at least one target trait comprises low
20 chill requirement.

38. The method of claim 36, wherein the plant of step (b) expresses both low chill requirement and disease/pest resistance as target traits.

39. The method of claim 38, wherein the disease/pest is selected from one or more of the following: bacterial leaf spot; Shaka (Plum pox virus); bacterial canker; root
25 rot; brown rot; peach canker; bacterial canker; bacterial blossom blight; nematode resistance.

40. The method of any one of claims 1 to 39, wherein the desired inheritable traits are selected from one or more of the group comprising: low chill requirement; high chill requirement; disease/pest resistance; fruit development period; fruit acidity; fruit
30 shape; fruit size; fruit flesh texture; fruit total solids (sugars); fruit pigmentation; fruit flesh pigmentation; fruit skin pubescence; stone adhesion to the fruit; tree habit; tree size; tree growth rate; spur morphology/ habit; pedicel length; pedicel thickness; suture presence/absence.

41. The method of any one of claims 1 to 40, wherein all the plants are of the
35 genus *Prunus*.

42. The method of claim 41, wherein the woody perennial plant of step (a) is selected from *Prunus persica*, *P. persica* var *nucipersica*, *P. persica* var *nectarina*, *P. salicina*, *P. avium*, *P. cerasus*, *P. domestica*, *P. amygdalus*, and *P. armeniaca*.

43. The method of claim 42, wherein the woody perennial plant of step (a) is a
5 peach or a nectarine variety.

44. The method of claim 41, wherein the one or more woody perennial plants of step (a) are sufficiently related to a peach or a nectarine so as to be able to hybridise with a peach or nectarine, but are of a different species to the one or more plants selected in step (b).

10 45. The method of any one of claims 41 to 44, wherein the one or more woody perennial plants of step (b) are selected from *Prunus persica*, *P. persica* var *nucipersica*, *P. persica* var *nectarina*, *P. salicina*, *P. avium*, *P. cerasus*, *P. domestica*, *P. amygdalus*, and *P. armeniaca*.

46. The method of any one of claims 41 to 44, wherein the one or more woody
15 perennial plants of step (b) are selected from peach or nectarine varieties.

47. The method of any one of claims 41 to 44, wherein the one or more woody perennial plants of step (b) are sufficiently related to a peach or a nectarine so as to be able to hybridise with a peach or nectarine, but are of a different species to the one or more plants selected in step (a).

20 48. The method of any one of claims 41 to 47, wherein step (d) further comprises one or more sequential back-crosses of one or more selected progeny plants with one or more parental woody perennial plants which are selected from peach, nectarine, plum cherry, almond or apricot varieties.

49. The method of claim 48, wherein the one or more parental woody
25 perennial plants are selected from peach or nectarine varieties.

50. The method of any one of claims 41 to 47, wherein step (d) further comprises one or more sequential back-crosses of one or more selected progeny plants with one or more woody perennial plants which are related to peaches, nectarines, plums or apricots, but of a different species to the plant selected in step (a), step (b) or both steps
30 (a) and step (b).

51. The method of any one of claims 1 to 50, wherein the woody perennial plant of step (a) is a peach or a nectarine, and the one or more woody perennial plants of step (b) are related to peaches or nectarines, but are of a different species.

52. The method of claim 51, wherein step (d) further comprises one or more sequential back-crosses of one or more selected progeny plants with one or more parental woody perennial plants which are selected from peach or nectarine varieties.

53. The method of claim 52, wherein the first and third woody perennial plants
5 are the same, or of the same variety.

54. The method of any one of claims 41 to 53, wherein the one or more target traits comprise low chill requirement.

55. The method of claim 54, wherein the one or more woody perennial plants of step (b) also comprise disease/pest resistance, as an inheritable trait.

10 56. The method of any one of claims 41 to 45, or 48 or 55, wherein the one or more woody perennial plants of step (b) are selected from plum or apricot varieties.

57. The method of claim 56, wherein step (d) further comprises one or more sequential back-crosses of one or more selected progeny plants with the pollen of a plurality of woody perennial plants which are selected from plum or apricot varieties.

15 58. The method of any one of claims 1 to 57, wherein the resulting woody perennial breeding line is male sterile, homozygous for the desired set of inheritable traits, and homozygous for at least one allele associated with a target trait.

59. A male sterile woody perennial plant breeding line comprising a target inheritable trait, generated by a method according to any one of claims 1 to 58.

20 60. The male sterile woody perennial plant breeding line of claim 59, which is a *Prunus* variety selected from peach, nectarine, plum, cherry, apricot or almond varieties.

61. The male sterile woody perennial plant breeding line of claim 59, wherein the cross of step (c) is an interspecific cross.

25 62. The male sterile woody perennial plant breeding line of claim 61, which is a plumcot, or a pleach.

63. The male sterile woody perennial plant breeding line of claim 61, which is essentially a *Prunus* variety selected from peach, nectarine, plum, cherry, apricot or almond varieties.

30 64. The male sterile woody perennial plant breeding line of any one of claims 61 to 63, wherein the desired inheritable traits are selected from one or more of the group comprising: low chill requirement; high chill requirement; disease/pest resistance; fruit development period; fruit acidity; fruit shape; fruit size; fruit flesh texture; fruit total solids (sugars); fruit pigmentation; fruit flesh pigmentation; fruit skin pubescence; stone adhesion to the fruit; tree habit; tree size; tree growth rate; spur morphology/ habit;
35 pedicel length; pedicel thickness; suture presence/absence.

65. The male sterile woody perennial plant breeding line of claim 64, wherein the one or more target traits comprise low chill requirement, disease/pest resistance, or both.

66. The male sterile woody perennial plant breeding line of any one of claims 5 61 to 65, which is male sterile, homozygous for the desired set of inheritable traits, and homozygous for at least one allele associated with a target trait.

67. A method for generating a woody perennial variety comprising one or more target inheritable traits, comprising crossing a first selected woody perennial plant variety with a desired set of inheritable traits, or a group of plants sharing a set of desired 10 inheritable traits, with a second selected woody perennial plant which is homozygous for male sterility, and homozygous for one or more target traits.

68. The method of claim 67, wherein the second plant is of known genotype with respect to the desired set of inheritable traits of the first selected woody perennial plant or group of plants sharing a set of desired inheritable traits.

69. The method of claim 67 or 68, wherein the second plant expresses the 15 desired set of inheritable traits of the first selected woody perennial plant or group of plants sharing a set of desired inheritable traits.

70. The method of claim 67 or 68, wherein the second plant is homozygous for one or more of the desired set of inheritable traits of the first selected woody perennial 20 plant or group of plants sharing a set of desired inheritable traits.

71. The method of claim 67 or 68, wherein the second plant is homozygous for each of the desired set of inheritable traits of the first selected woody perennial plant or group of plants sharing a set of desired inheritable traits.

72. The method of any one of claims 67 to 71, wherein the second woody 25 perennial plant is a male sterile woody perennial plant breeding line according to any one of claims 59 to 66.

73. The method of any one of claims 67 to 72, wherein the first selected woody perennial plant variety with a desired set of inheritable traits, or group of plants sharing a set of desired inheritable traits, are planted in an orchard, surrounding the 30 second plant.

74. The method of any one of claims 67 to 73, wherein the flowers of the second plant are artificially fertilised with pollen from the first selected woody perennial plant variety with a desired set of inheritable traits, or a mixture of pollen from the group of plants sharing a set of desired inheritable traits.

75. The method of any one of claims 67 to 74, wherein all of the plants are of the *Prunus* genus.

76. The method of claim 75, wherein the plants are selected from *Prunus* plants which are, or are essentially peach, nectarine, plum, cherry, apricot or almond varieties.

77. The method of claim 75, wherein first selected woody perennial plant variety with a desired set of inheritable traits, or group of plants sharing a set of desired inheritable traits, or the second selected woody perennial plant, is selected from hybrids between two different *Prunus* species.

78. A woody perennial plant variety generated by the method of any one of claims 67 to 77.

79. The woody perennial plant variety of claim 78, which is selected from *Prunus* plants which are, or are essentially peach, nectarine, plum, cherry, apricot or almond varieties.

80. The woody perennial plant variety of claim 78, which is selected from *Prunus* plants which are, or are essentially peach or nectarine varieties.

81. The woody perennial plant variety of any one of claims 78 to 80 which expresses one or more target traits selected from the group comprising: low chill requirement; high chill requirement; disease/pest resistance; fruit development period; fruit acidity; fruit shape; fruit size; fruit flesh texture; fruit total solids (sugars); fruit skin pigmentation; fruit flesh pigmentation; fruit skin pubescence; stone adhesion to the fruit; tree habit; tree size; tree growth rate; spur morphology/ habit; pedicel length; pedicel thickness; suture presence/absence.

82. The woody perennial plant variety of claim 81, which expresses at least low chill, disease/pest resistance, or both as target traits.

83. The woody perennial plant variety of claim 81 or claim 82, which expresses heterosis for one or more target traits.